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ECOLOGY AND THERMAL INACTIVATION OF MICROBES  
IN AND ON INTERPLANETARY SPACE VEHICLE  
COMPONENTS

Thirty-ninth Quarterly Report of Progress

Order No. W-13411

October 1, 1974 - December 31, 1974

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## ECOLOGY AND THERMAL INACTIVATION OF MICROBES IN AND ON INTERPLANETARY SPACE VEHICLE COMPONENTS

### Introduction

The dry-heat terminal sterilization cycle for the Viking lander was tentatively set for a total of 60 hr. This provides 112 C exposure for 30 hr with 1.2 µg of water per ml of head-space air (25% RH at 0 C at STP). These parameters were based on experimental findings by several National Aeronautics and Space Administration laboratories' studies of dry-heat inactivation studies of Bacillus subtilis var. niger spores. Recent studies have shown that naturally-occurring spores found around assembly and industrial manufacturing areas of space vehicles exhibited higher dry-heat resistance than Bacillus subtilis var. niger (Bond, et al., 1970; 1973). Studies from the Kennedy Space Center Teflon ribbon experiments showed recovery of spores after the terminal sterilization cycle at 113 C and 0.134% RH (25% RH at 0 C STP). However, a number of studies suggested that resistance to heat in spores that occur naturally should be different from that of spores produced in the laboratory media (Curran, 1935; Vinton et al., 1947; Cameron et al., 1936, and others).

In the thirty-seventh quarterly report we have demonstrated that media-sporulated spores (of three isolates from Cape Kennedy Teflon ribbon experiments) showed significant numbers of survivors after 30 hr at 113 C and 0.134% RH (25% RH at 0 C STP).

(See Figure 1 in this report.) The data suggest these spores were still very heat resistant.

In this quarter we extended our investigation on the dry heat resistance of microorganisms in soil samples obtained from other areas in the United States. From these experiments, isolated colonies (microbial survivors from extended heat exposure) were picked from agar plates and subcultured for identification and for the production of spores for dry-heat resistance studies.

## I. EXPERIMENTAL

### A. Soil samples

We have received soil samples from four geographical areas of the United States: Denver, Colorado; Pasadena, California; Kennedy Space Center, Florida; and Cincinnati, Ohio. Except for the Cincinnati soil sample, all samples were collected near industrial manufacturing and assembly areas for space vehicles. All soil samples were thoroughly mixed and processed through a brass sieve series down to 0.250-mm screen size (U. S. Standard, #60 mesh). This procedure eliminated the larger rocks, twigs, roots, and shell particles. All soil samples were placed in clean, screw-cap Mason jars and stored at ambient temperature. For all the experiments, a 0.5-gm sample was weighed in a stainless steel cup.

## B. Heat exposure methods

### 1. Kennedy Space Center terminal sterilization process

In October 1974, a Cincinnati employee was sent to the Kennedy Space Center, JPL Bioassay Laboratory to perform dry-heat inactivation experiments on Kennedy Space Center and Cincinnati soil samples. The experiment was done at this laboratory because the sterilization facility is equipped to simulate conditions that will be encountered during terminal sterilization of space vehicles. The test fixtures are stainless steel cups similar to those used in our laboratory. A description of the process is given in the 24th and 30th quarterly reports.

Eighteen 0.5-gm samples of each soil type were exposed to the heating cycle which provided an exposure of 30 hr at 112 C with 1.2  $\mu$ g of water per ml of headspace air. A nitrogen gas containing the specified concentration of water was used, and a moisture analyzer monitored the gas flowing over the soil samples in the oven.

### 2. Cincinnati dry-heat process

Soil samples obtained from different geographical areas were weighed in 0.5-gm amounts into stainless cups, placed in glass petri dishes and dried in a vacuum oven at 50 C for 1 hr. The sample cups containing soil were placed in a forced-air oven, which had been set at the desired

temperature and which provided a relative humidity of less than 1%. Thermocouples were installed in the oven and temperature was monitored by a Bristol temperature recorder throughout the study.

#### C. Recovery methods

For the KSC run, sample cups containing soil were removed from the oven and aseptically placed in 25 x 150-mm sterile, screw-cap tubes under a Class 100 vertical flow hood. These samples were transported to the Cincinnati Food Research Laboratory for recovery studies. Ten ml of sterile 1% peptone water was added to each tube, and these tubes were sonified for 24 min. The samples were plated by the conventional pour-plate method using TSA supplemented with 0.1% soluble starch and 0.2% yeast extract. The plates were incubated for 5 days at 35 C prior to counting. For the Cincinnati dry-heat studies, the same recovery procedure was used.

## II. RESULTS AND DISCUSSION

A series of experiments was conducted to determine the dry-heat resistance of microorganisms in soil obtained from four geographical areas of the United States.

The results of the KSC terminal sterilization cycle experiment are shown in Figure 2. The average number of viable organisms per ml was calculated for 18 replicate soil samples for each sample area and points plotted equivalent to

30-hr exposure at 112 C. The results showed a reduction of 3 logs from the initial population for both KSC and Cincinnati soil samples. A statistical analysis of the  $\log_{10}$  number of viable organisms resulted in a variance of the mean of 0.00044 for the Cincinnati soil samples and 0.00109 for KSC soil samples, and their 99% confidence limits are shown in Table 1. The number of surviving organisms was plotted for the Cincinnati soil samples (Fig. 2) based on the Cincinnati and KSC dry-heat inactivation methods. The results of both methods are in close agreement.

Results on the dry-heat resistance of microorganisms on six soil samples obtained from four geographical areas of the United States are given in Figures 3-8. In all six soil samples examined, microbial survivors were present after 60-hr exposure at 112 C. Survival curves at 112 C showed a rapid decline in the number of viable organisms (1- to 3-log reduction) in the first 6 to 12 hr, followed by a slower decline at the latter stages of heat exposure.

A similar pattern was observed on survivor curves at 125 C; however, the rapid decline of viable organisms occurred in the first 6 hr of heat exposure. At 125 C, no viable organisms were detected after 36 hr for both Colorado soil samples; 54 hr for Bldg. 103, Pasadena soil sample; and 60 hr for Bldg. 179, Pasadena and Cincinnati soil samples. There are no data for the KSC soil sample at 125 C at this time.

Data from these studies suggest that highly resistant organisms are present in these soil samples. Although these organisms occur in low numbers (probably  $10^3$ /gm) in a soil sample, their presence should be considered in the design and establishment of dry-heat sterilization methods.



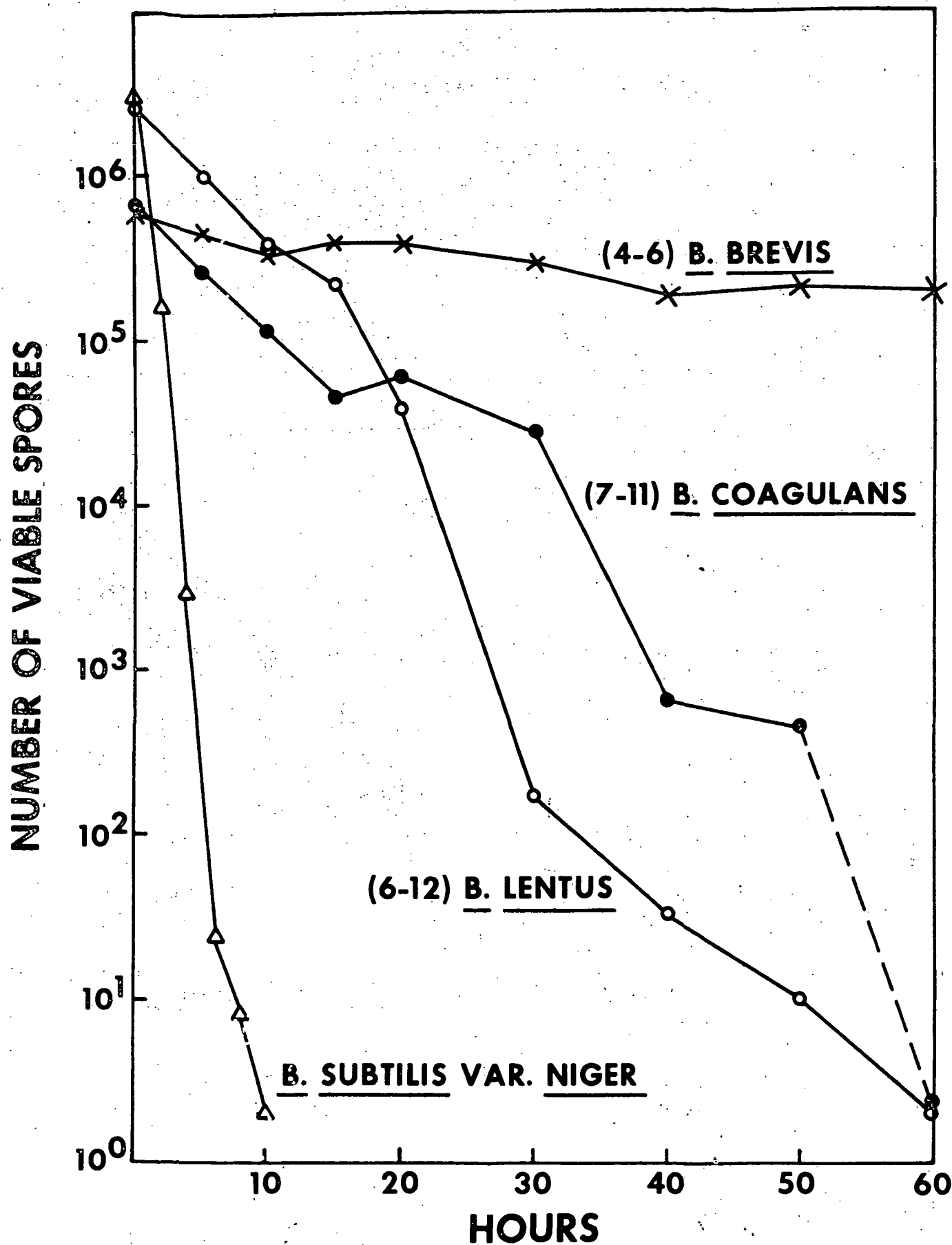
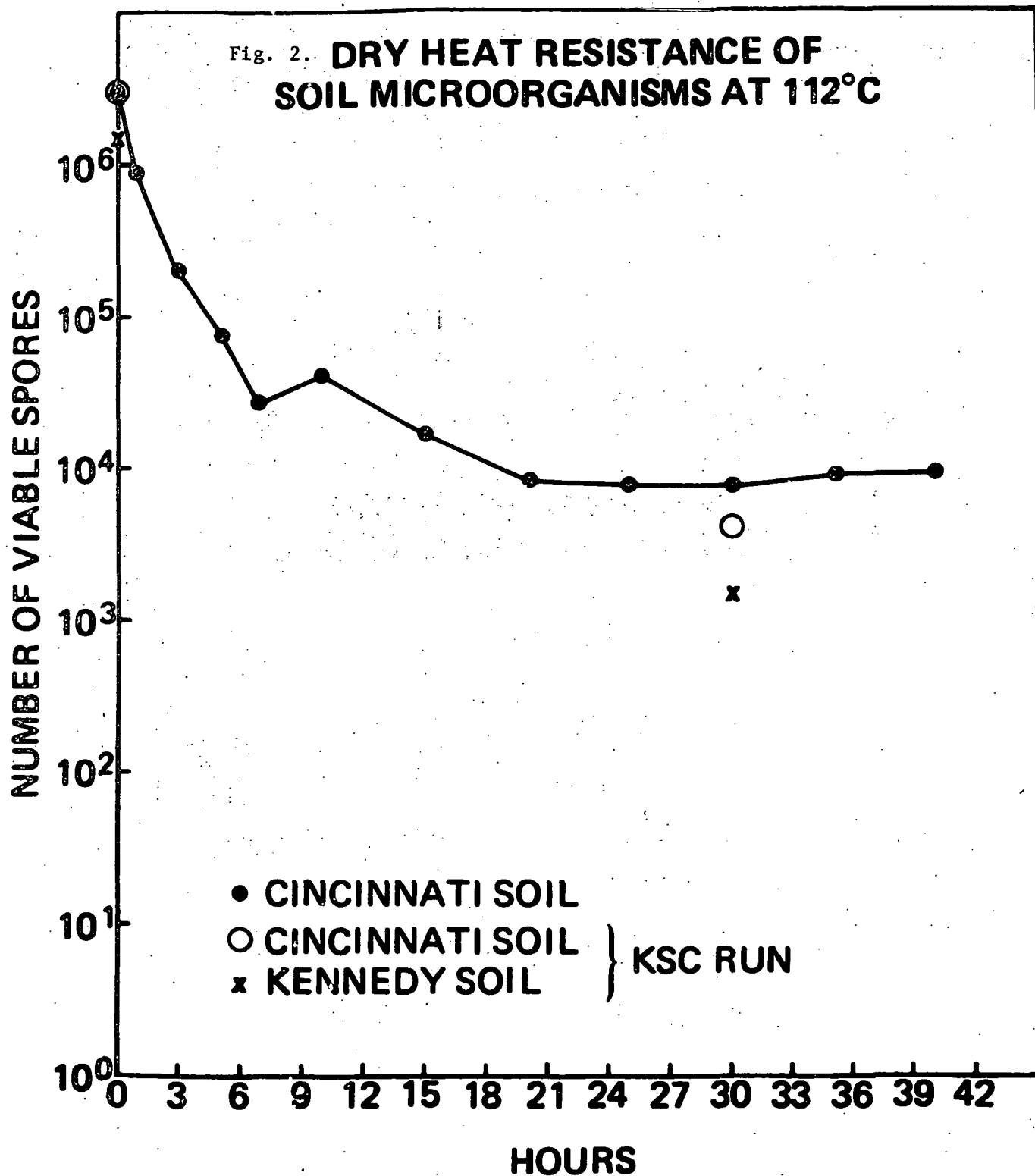
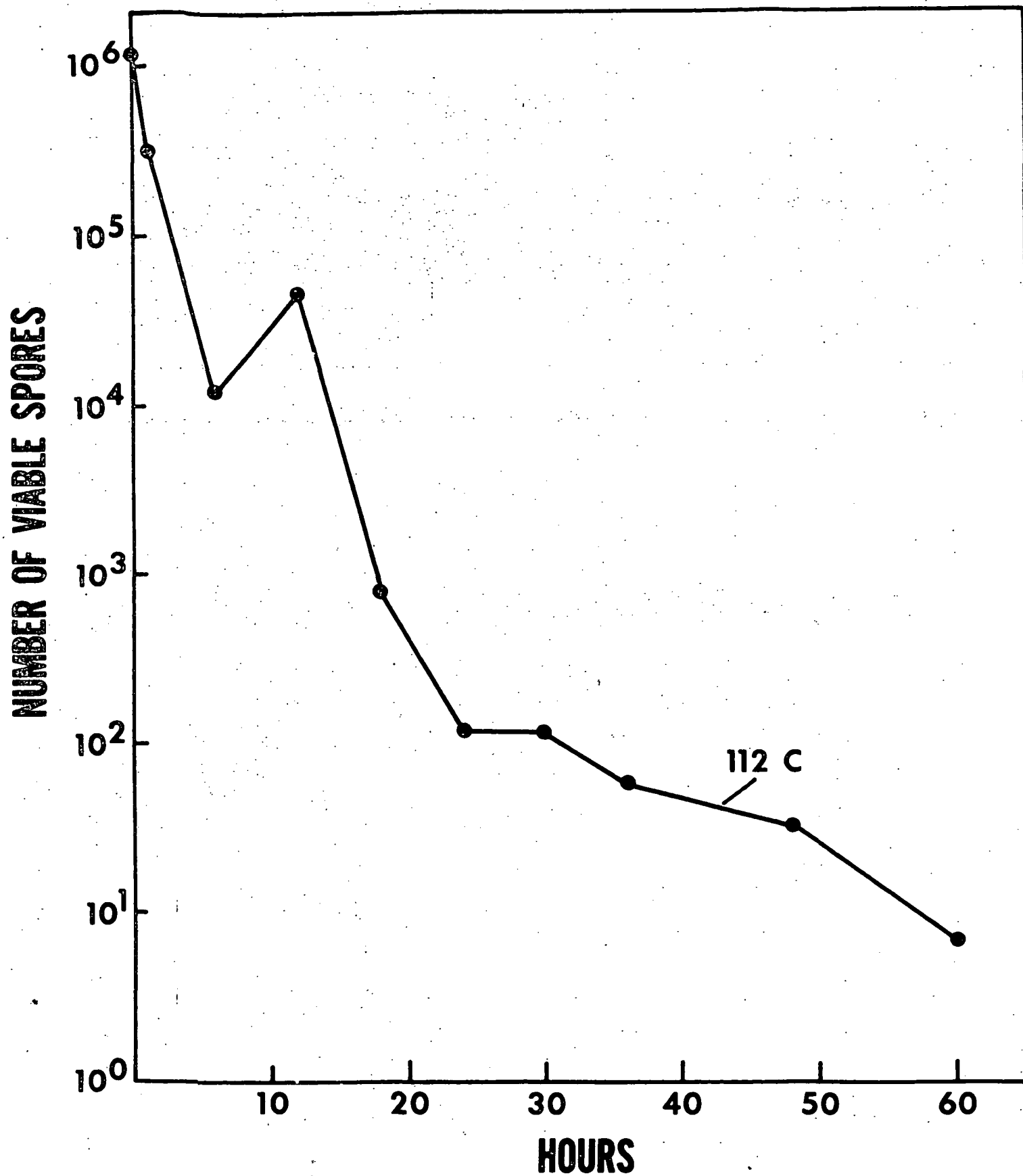


FIG. 1.-- THERMAL INACTIVATION OF SPORES 113 C AND 0.134% R.H (1.3  $\mu\text{g}$   $\text{H}_2\text{O}/\text{CM}^3$ )

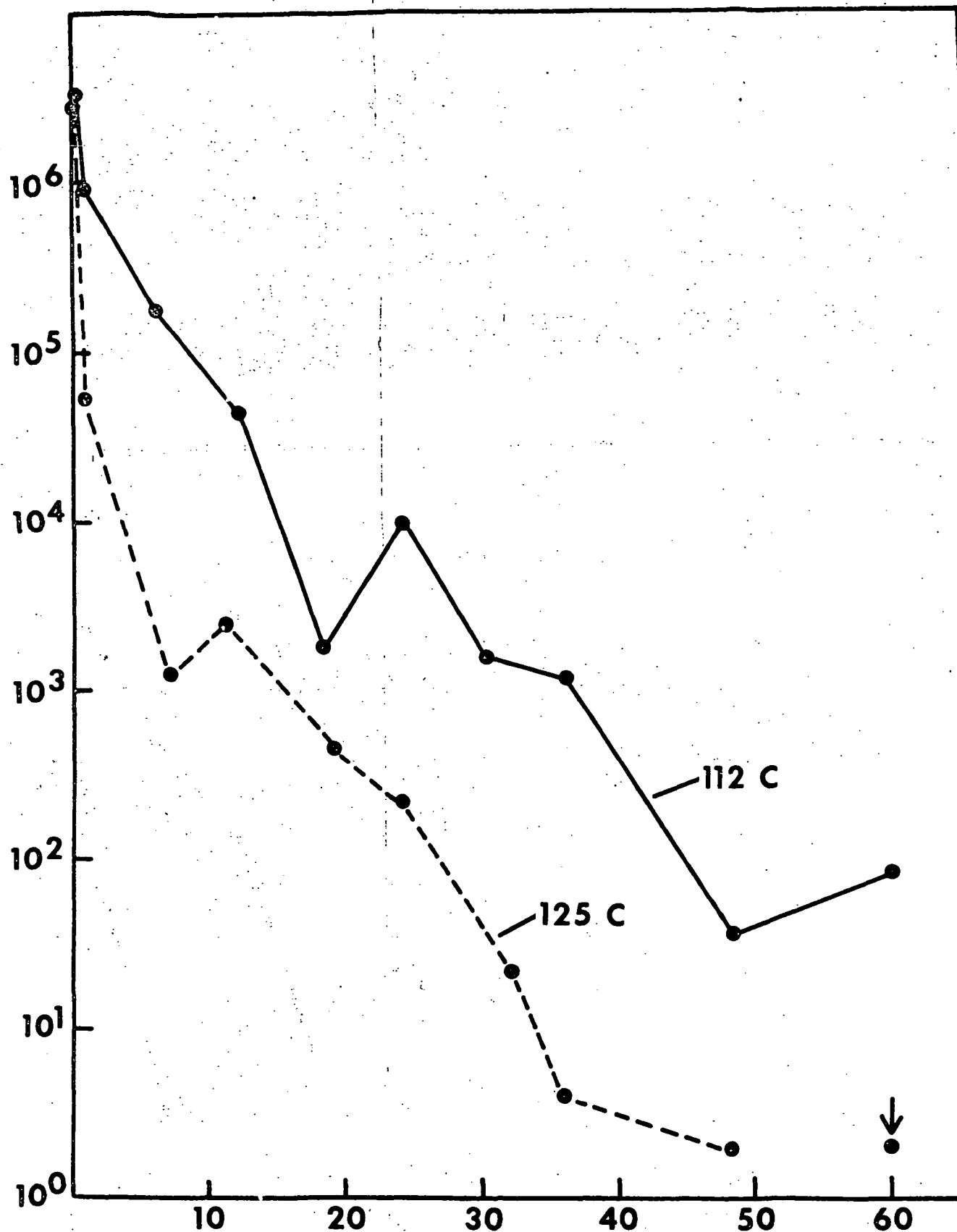
Fig. 2. DRY HEAT RESISTANCE OF  
SOIL MICROORGANISMS AT 112°C





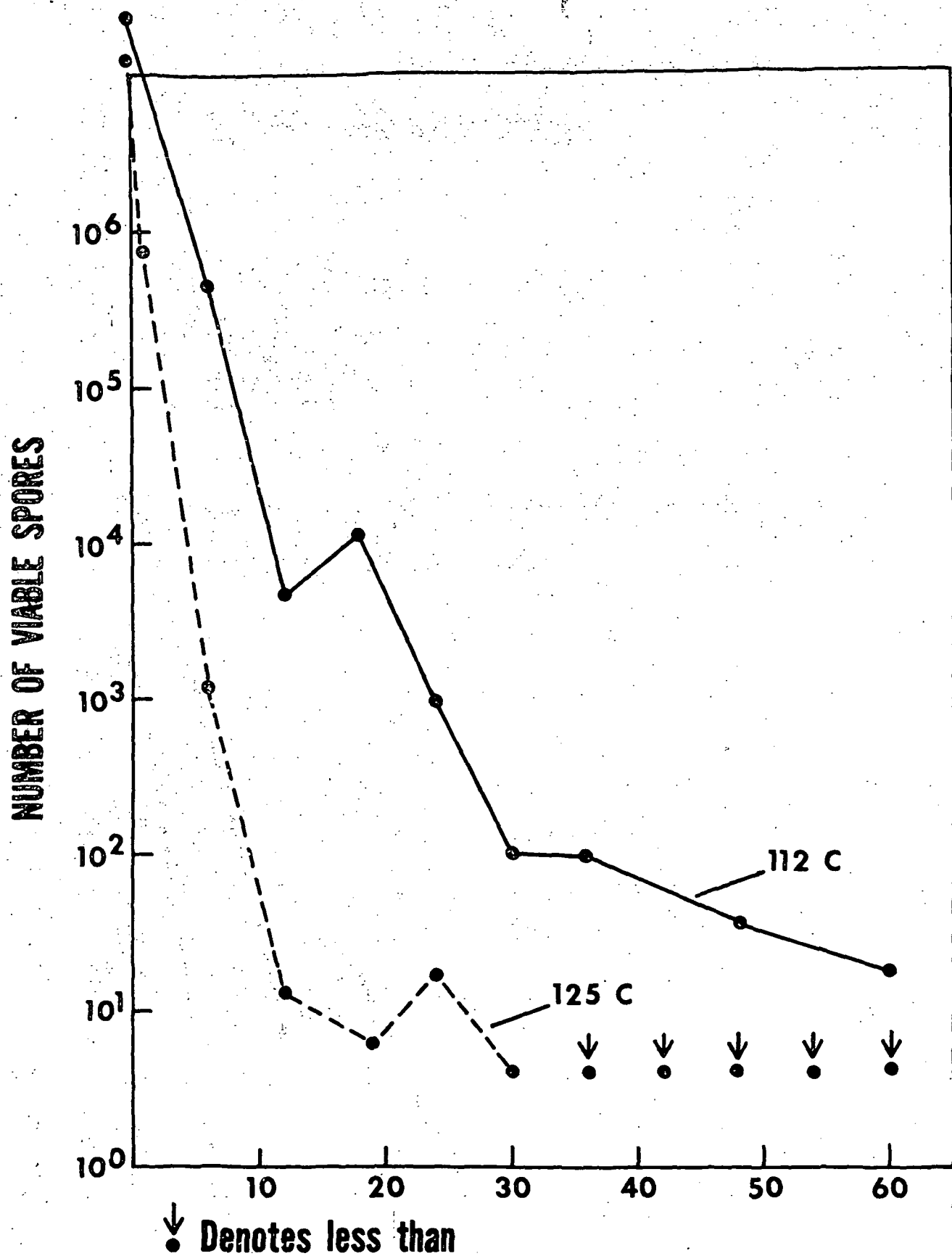
**FIG. 3 DRY-HEAT RESISTANCE OF SOIL MICROORGANISMS AT 112 C (KENNEDY SPACE CENTER SOIL SAMPLE)**

NUMBER OF VIABLE SPORES



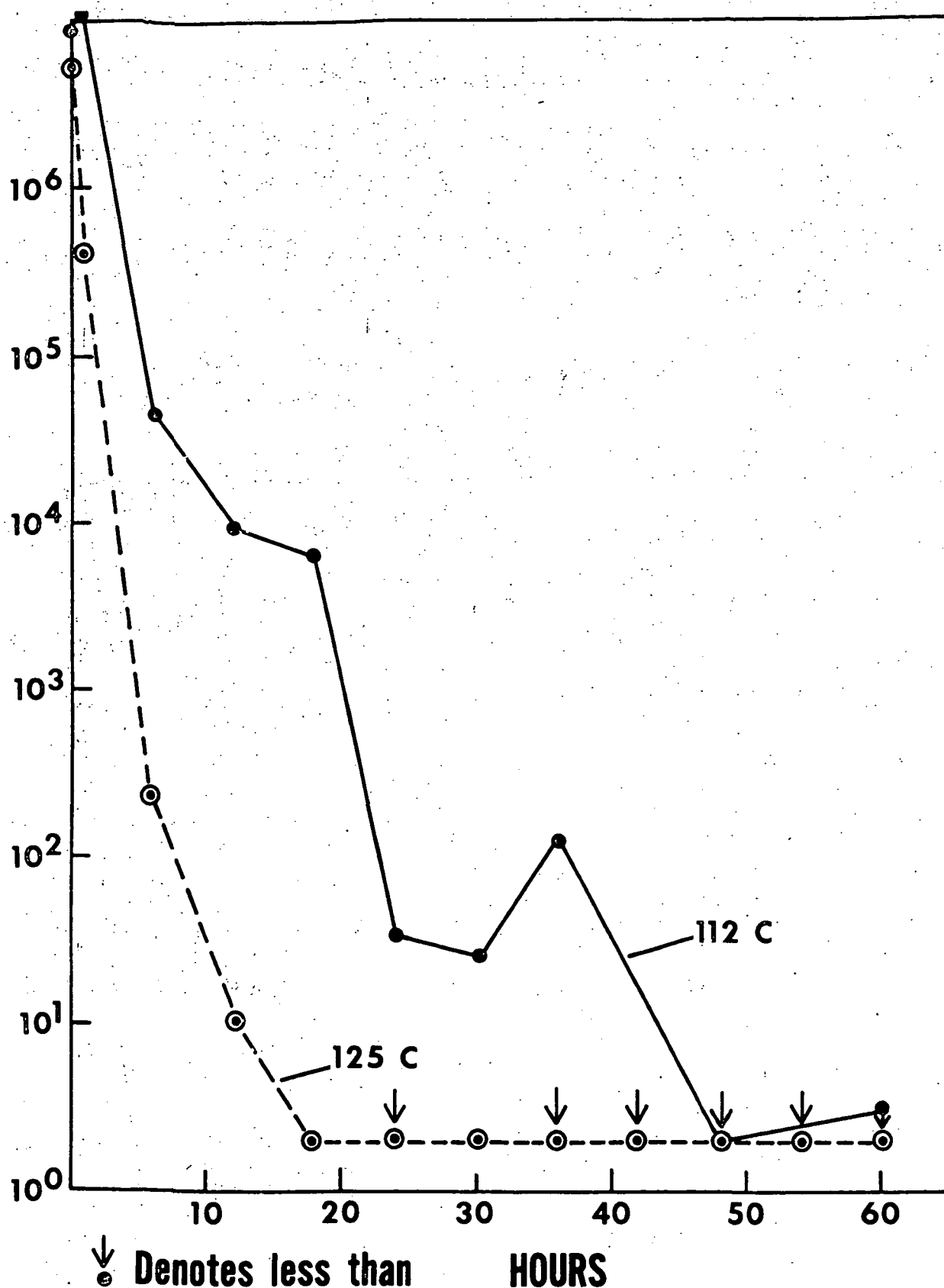
↓ Denotes less than HOURS

FIG. 4 DRY-HEAT RESISTANCE OF SOIL MICROORGANISMS  
AT 112 AND 125 C (CINCINNATI, OHIO SOIL SAMPLE)



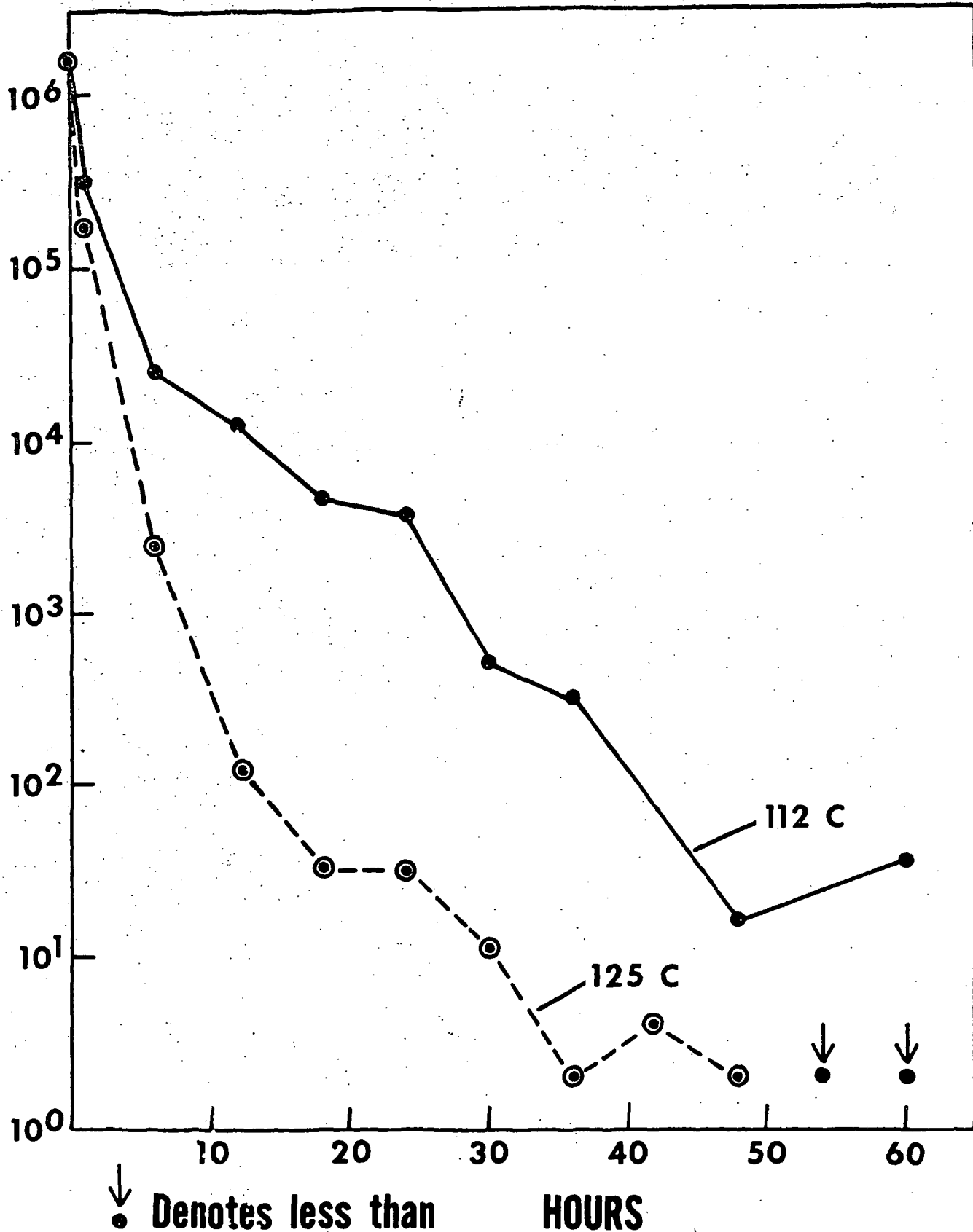
**FIG. 5 DRY-HEAT RESISTANCE OF SOIL MICROORGANISMS AT 112 AND 125 C (DENVER, COLORADO, LOADING DOCK, SSB SOIL SAMPLE)**

NUMBER OF VIABLE SPORES



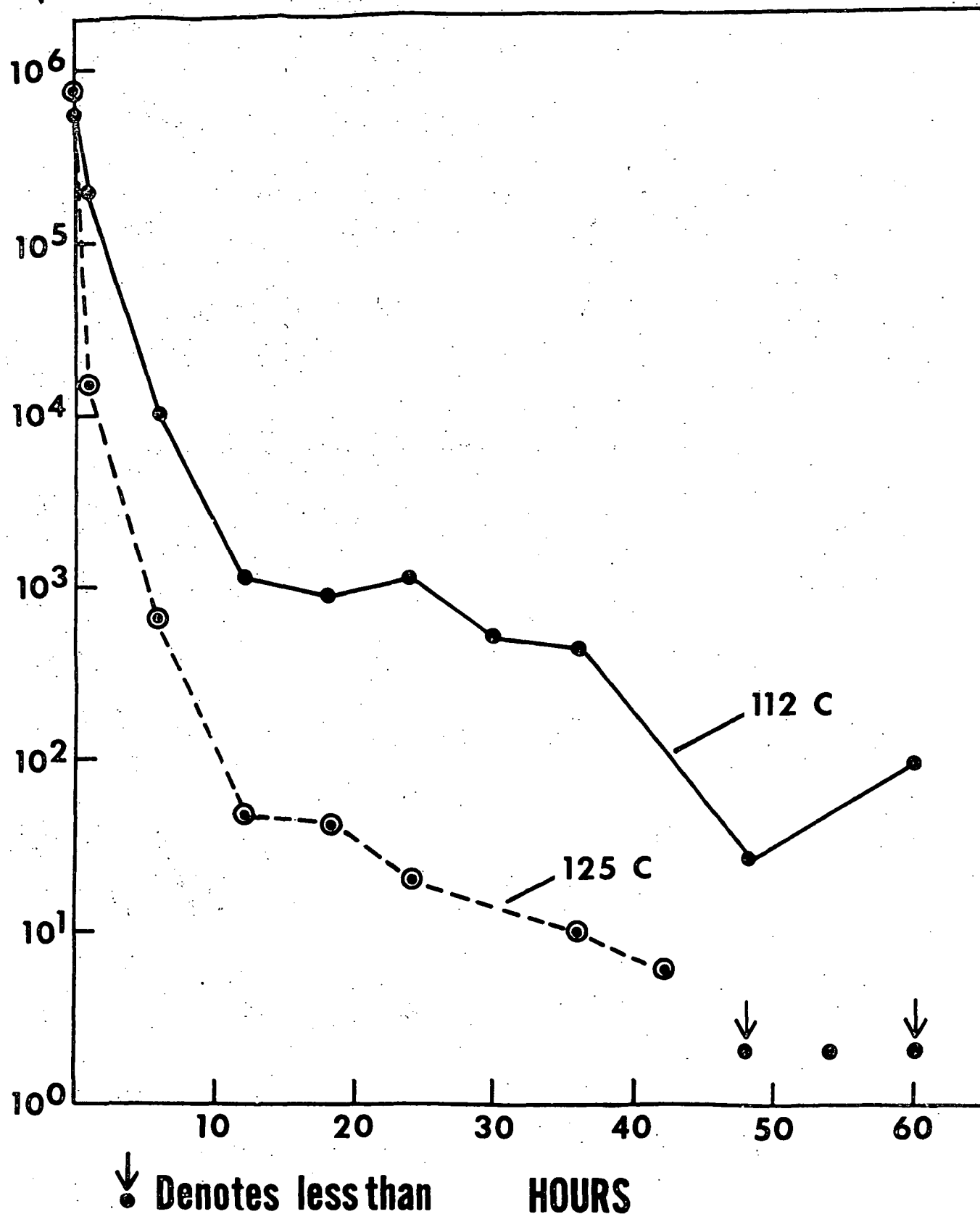
**FIG. 6 DRY-HEAT RESISTANCE OF SOIL MICROORGANISMS  
AT 112 AND 125 C (DENVER, COLORADO, HILL  
BEHIND, SSB SOIL SAMPLE)**

NUMBER OF VIABLE SPORES



**FIG. 7 DRY-HEAT RESISTANCE OF SOIL MICROORGANISMS  
AT 112 AND 125 C (PASADENA, CALIFORNIA, BLDG.  
103 SOIL SAMPLE)**

NUMBER OF VIABLE SPORES



**FIG. 8 DRY-HEAT RESISTANCE OF SOIL MICROORGANISMS  
AT 112 AND 125 C (PASADENA, CALIFORNIA, BLDG.  
179 SOIL SAMPLE)**